First International Conference on Tattoo Safety

Berlin 2013

Microencapsulation of Dyes and Pigments

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Surface Layers → SURFLAY

Content

1. Layer by Layer (LbL) Technology
2. Encapsulation of Tattoo pigments
3. Biocompatibility
4. Possible solutions of Tattoo problems by LbL

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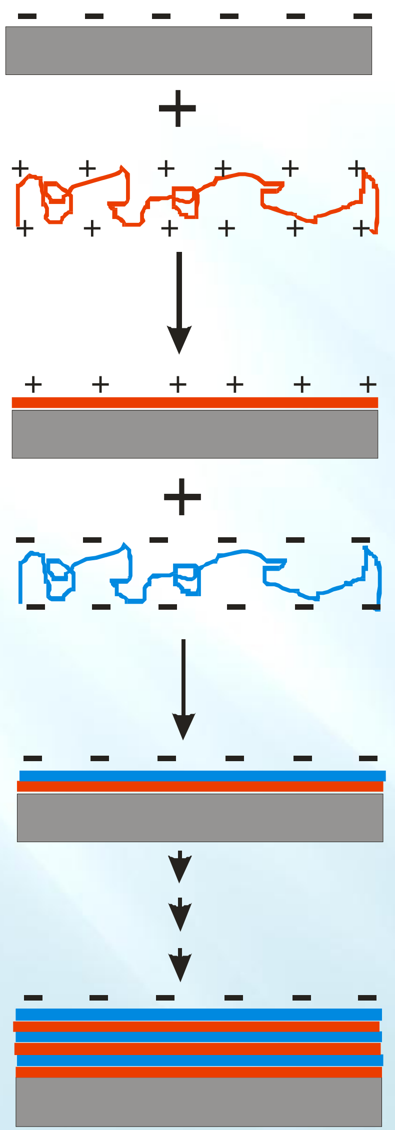
Layer by Layer (LbL-technology)

G. Decher 1991, reviewed in Science 277 (1997) 1232



Charged Substrate (planar, surface structured, colloidal, porous)

Polycation in excess, aqueous solution 1g/l,
Control of pH, ion strength

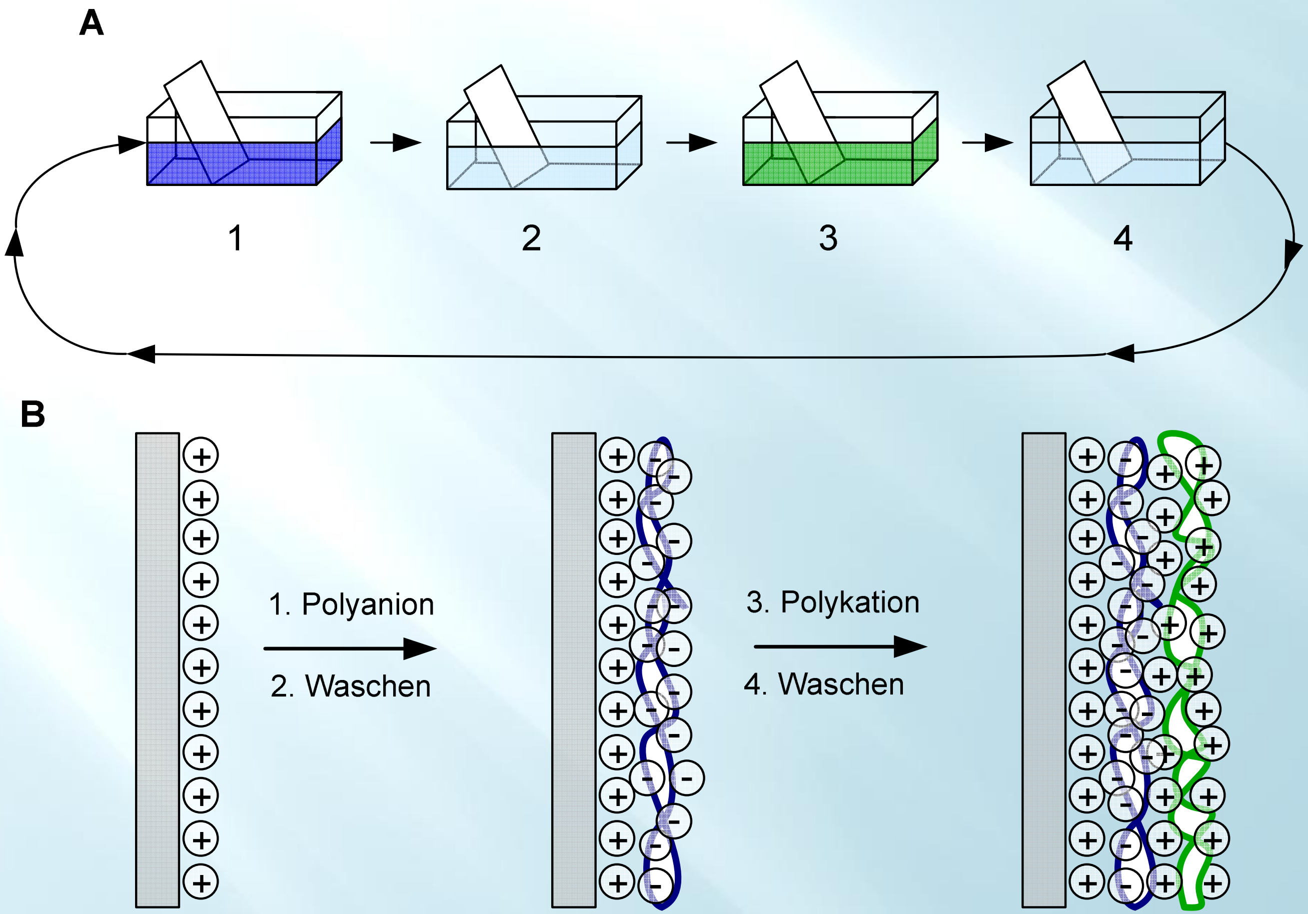
Self-limited adsorption, charge reversal
(ζ-potential + 30-60 mV), removal excess polyelectrolyte

Polyanion in excess

Thickness per double layer
3 nm for PAH/PSS, ζ-potential -30 til -60 mV

Layer by Layer (LbL)
coated substrate

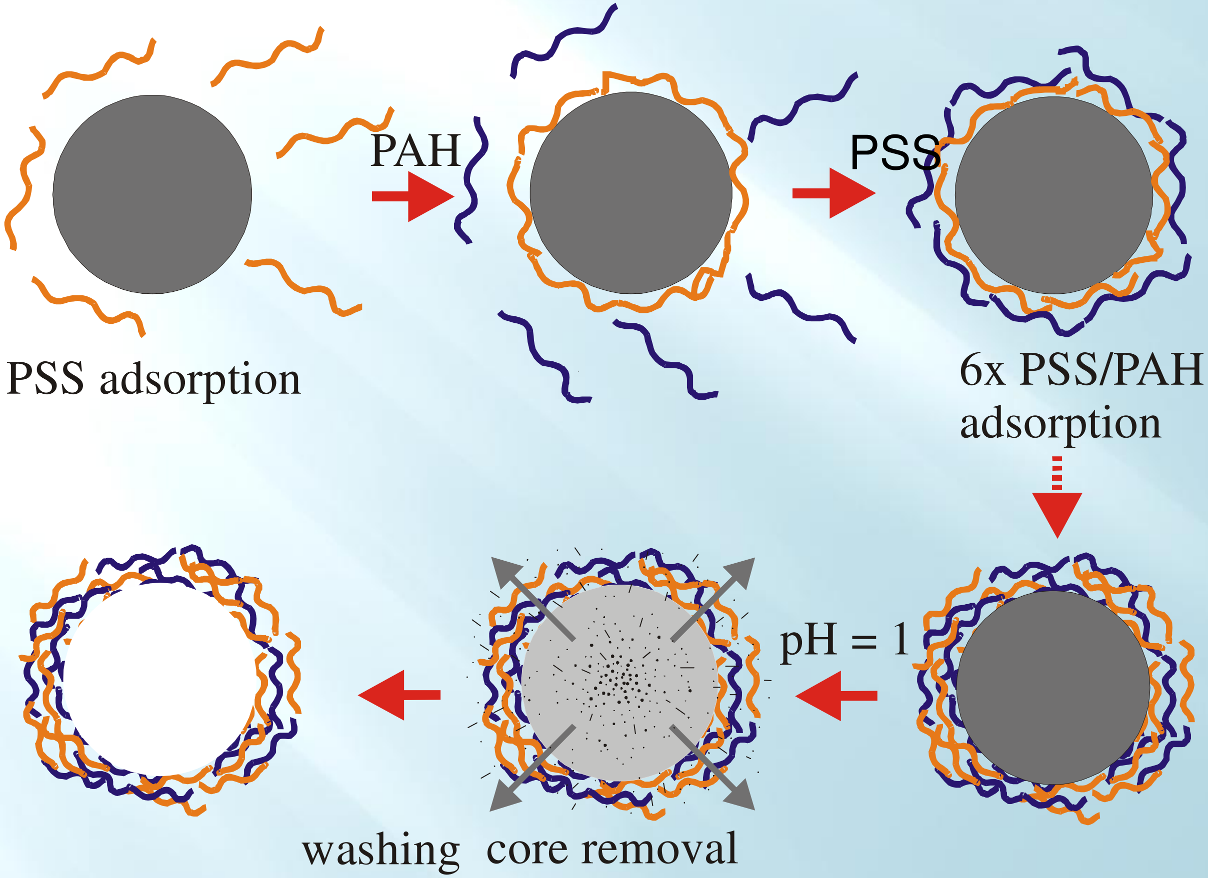
LbL-prozess



Large scale materials

- Dipping (minutes)
- Spraying (seconds)

Preparation of Microcapsules



Colloidal materials

- Centrifugation
- Filtration
- Sedimentation
- Dielectric separation

E. Donath, G. Sukhorukov, F. Caruso, A. Davis, H. Möhwald Angew. Chem. 110 (1998), 2324

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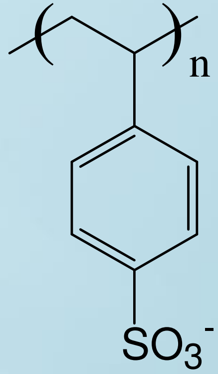
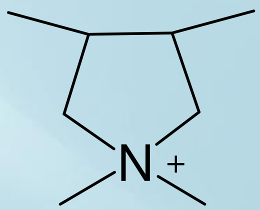
Coating materials

Polyelectrolytes (synthetic, natural)



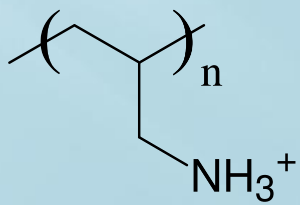
n = 350
Mw = 70 000

- **anionic:** Polystyrenesulfonate (PSS), PMAA etc.
  Alginate, DNA, Hyaluronic acid, …
  multivalent ions (phosphates, peptides)



Mw 200 000
Quaternary amines
pH independent

-**cationic:** Polydimethyldiallylamine,
  Polyallylamine (PAH)
  Chitosan
  multivalent ions (peptides, iron)



n = 700
Mw = 70 000

Primary amine
pH dependent
Coupling chemistry

- **amphoteric:** Proteins (enzymes, antibodies)

Nanoparticle materials

Instead of Polyelectrolyte electrostatically stabilized (charged) Nanoparticles (diameter 3-20 nm) alternating deposition with polyelectrolyte;

- biozide Ag, Au
- photoactive TiO_2
- magnetite Fe_3O_4
- catalytic Pd, Pt
- fluorescent CdSe, CdTe,

Advantages of LbL immobilized Nanoparticles

- Function mainly preserved
- stabilized against aggregation
- danger of nanoparticles removed by stable immobilization

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Polyelectrolyte Functionalization

Polyelectrolyte coupling chemistry

e.g. via carboxylate –COOH, amino $–NH_2$, or glycidyl

Functions e.g.:

- coloured, fluorescent,
- (bio)catalytic,
- (photo)reactive,
- sticking, selective adsorption
- releasing biozide, drugs, care materials
- hydrophobic, hydrophilic
- oligonucleotides (selective binding diagnostic)

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Multifunctionality

Each layer

- different material
- different functionality
- Interference of functionalities avoidable by intermediate dummy layers
- Defined surface, independent of material underneath

General properties of LbL-coatings

- **Thickness:** 1 – 5 nm (extreme 200 nm) controlled by
  - ion strength; polyelectrolyte material; layer number

- **Structure:** - „spaghetti“ type network
  - contain 20-60% water in wet state
  - mash size in nanometer range

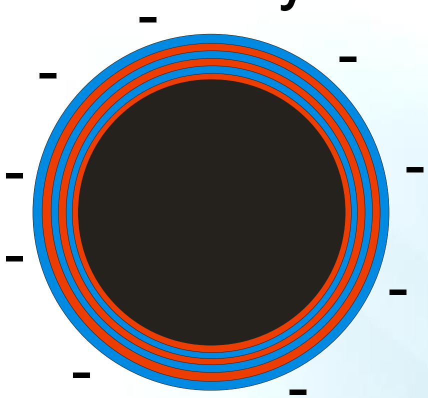
- **Stability:** tunable from very stable to soluble depending on
  - polyelectrolyte material
  - ion strength, pH, chaotropic salts, surfactants

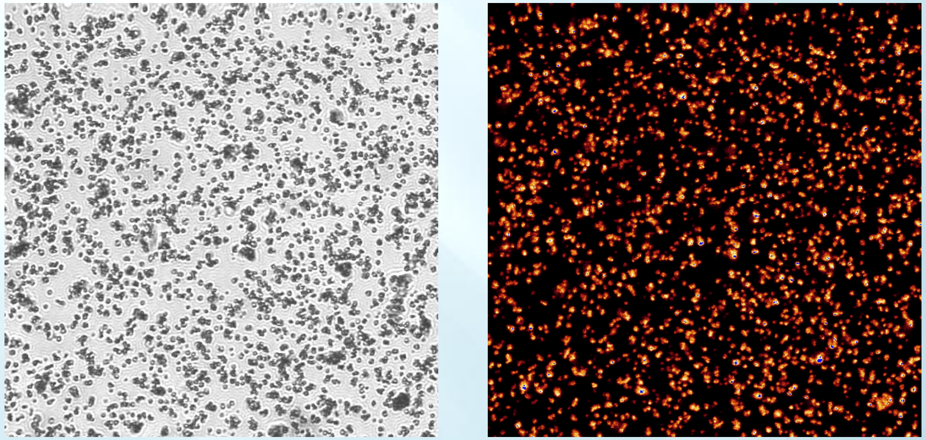
General properties of LbL-coatings

- **Charge:** - surface either positive or negative,
  - interior usually neutral,
  - upcharging by pH shift (weak polyelectrolytes)
  - control of permeability (release properties)
- **Permeability:** - semipermeable, cut off controllable > 1 kD
  - switching by external trigger possible
  - water and monovalent salts go through
  - impermeable for enzymes, nanoparticles, RNA
- **Nanoroughness:** - surface fuzzy and rough (+/- 2 nm, dry state)
  - coupling especially efficient (enzymes, DNA)

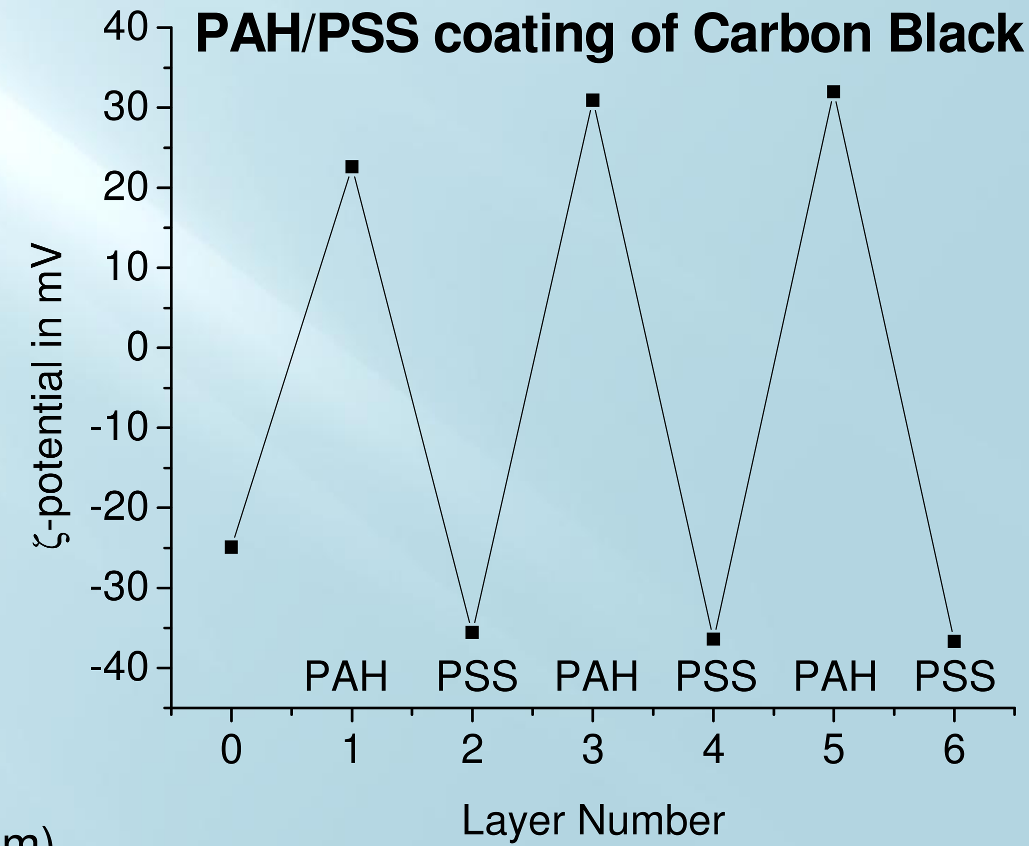
2. Encapsulation of Tattoo pigments (MTDerm)

3 double layers Polyallylamine-Rho/ Polystyrene sulphonate





Confocal Laser Scanning Microscopy (40 x 40 µm)
Transmission (Core TiO_2)   Fluorescence (Layer)

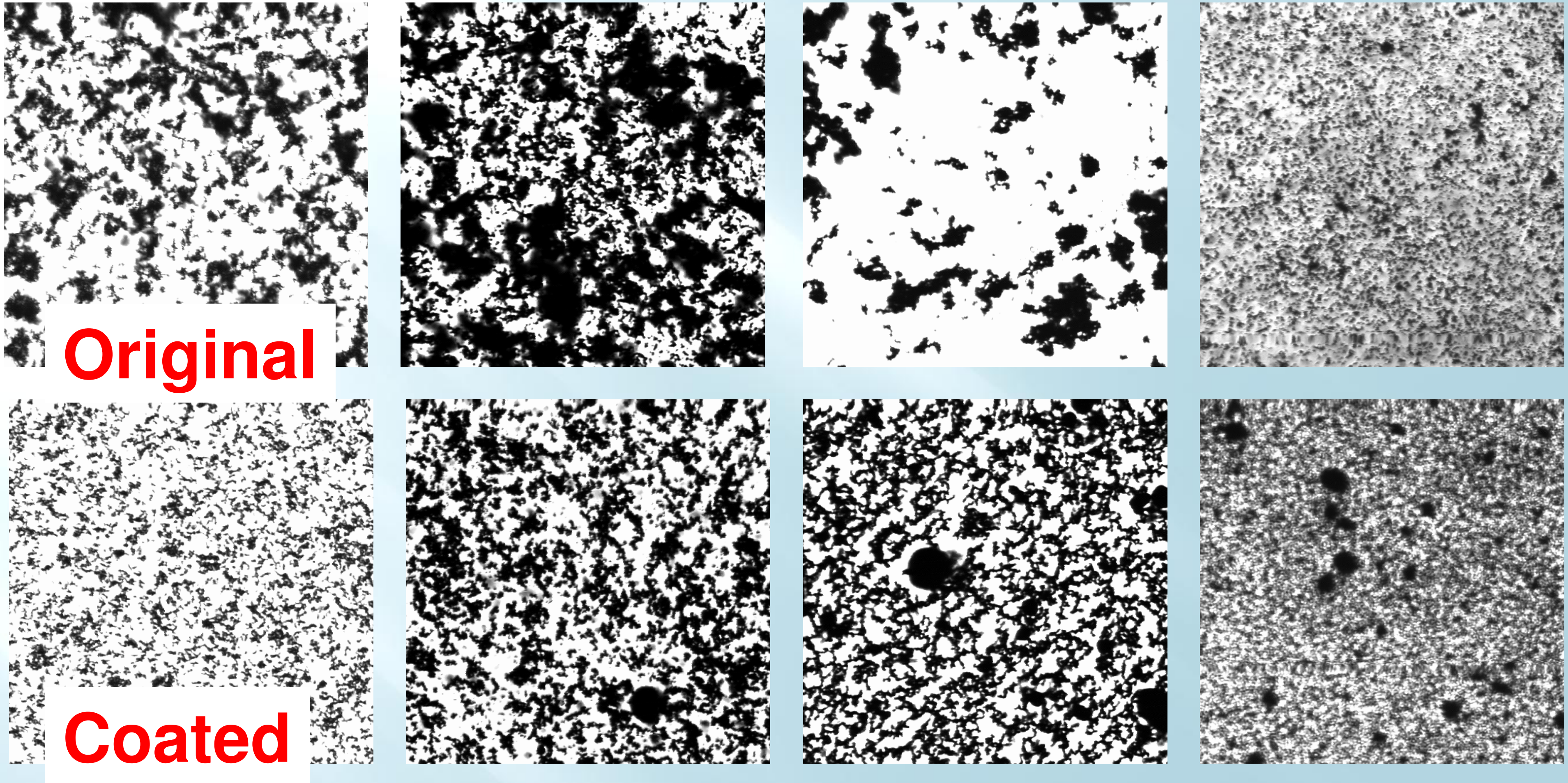


Change of ζ-potential

Unification of Surface potential by 4L coating

	Zeta-Potential, mV)	
	Before coating	after coating
1. Titanium dioxide	- 47	- 35
2. Iron oxide yellow	- 29	- 34
3. Iron oxid red	- 32	- 35
4. Iron oxid black	- 29	- 37
5. Carbon black	- 24	- 36
6. FD&C Yellow 6 lake	- 18	- 31
7. D$C Red 30 lake	- 30	- 30
8. Pigment Red 5	- 25	- 31
9. FD&C Red 40 lake	+ 22	- 36
10. Pigment Blue 15	- 30	- 32

Separation/Aggregation stability of Pigments



Iron oxide yellow iron oxide red carbon black titanium dioxide

Transmission image 40 µm x 40 µm

3. Biocompatibility of LbL-coatings

RKO Cells 24 h in Suspension 20 g/l Particles (diameter 1 µm) without shaking

Number	*Core / Coating*	*% cell survival*
1 pure	TiO_2	0
2	TiO_2/PAH/PSS/PAH/PSS	0
3	TiO_2/PAH/PSS/PAH/PMAA	0
4	TiO_2/PAH/PSS/PAH/Nafion	0
5	TiO_2/PAH/PSS/PAH/PVPho	0
6 cationic	TiO_2/PAH/PSS/PAH	0
7 pure	SiO_2	99
8	SiO_2/PAH/PSS/PAH/PSS	92

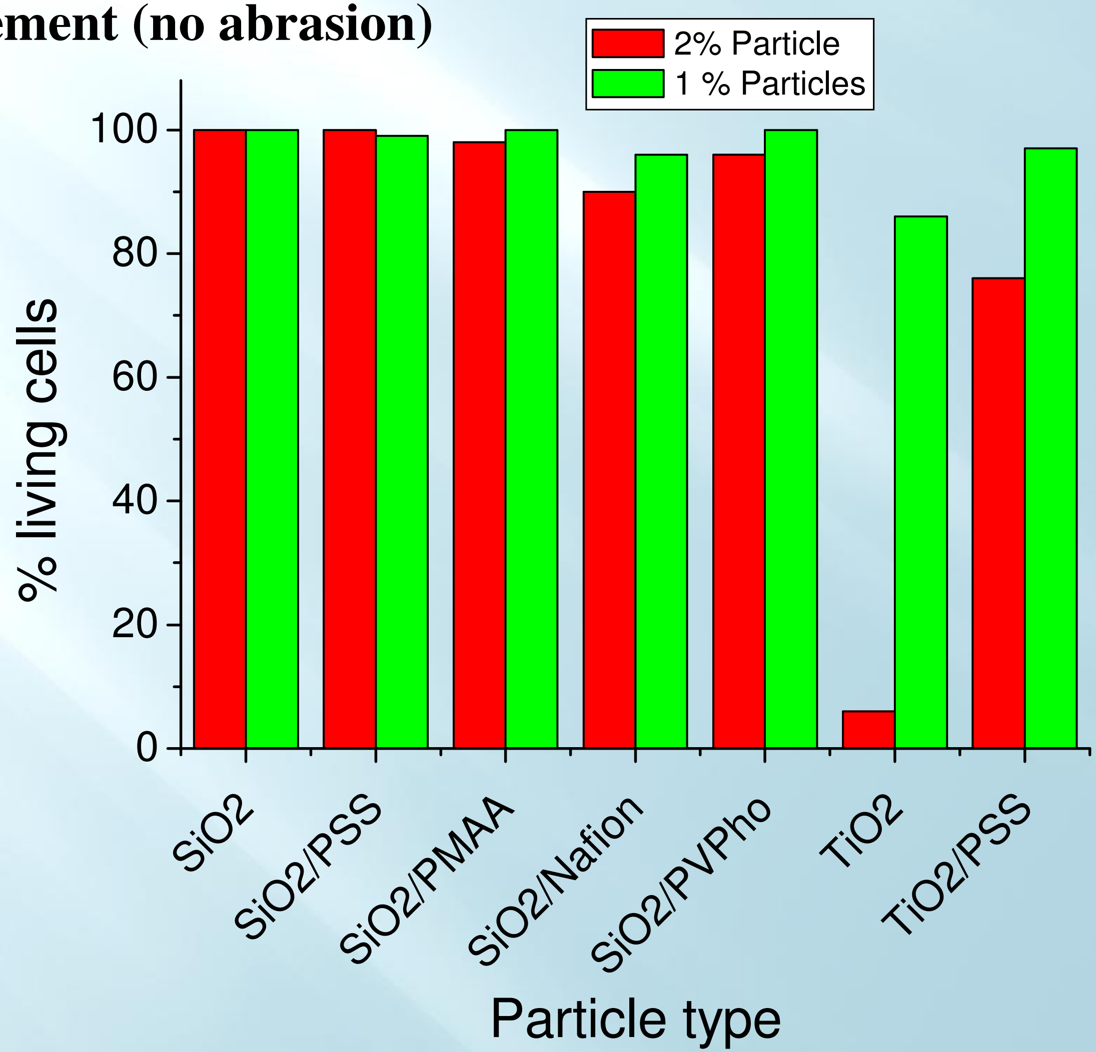
PAH: Polyallylamine
PSS: Polystyrenesulphonate
PMAA: Polymethacrylic acid
Nafion: sulphonated Teflon (perfluorated/hydrophob)
PVPh: Polyvinylphosphate

Density $SiO_2 = 1.8\ g/cm^3$
$TiO_2 = 4.3\ g/cm^3$

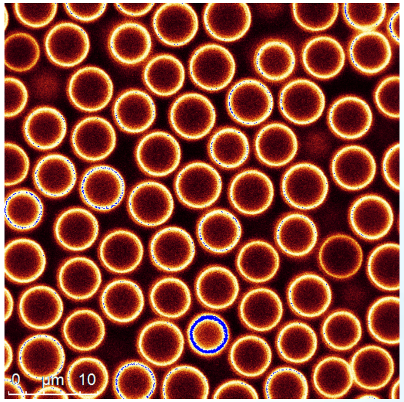
Cells are killed by pressure or by suffocating!

Cytotoxicity of LbL-coated particles

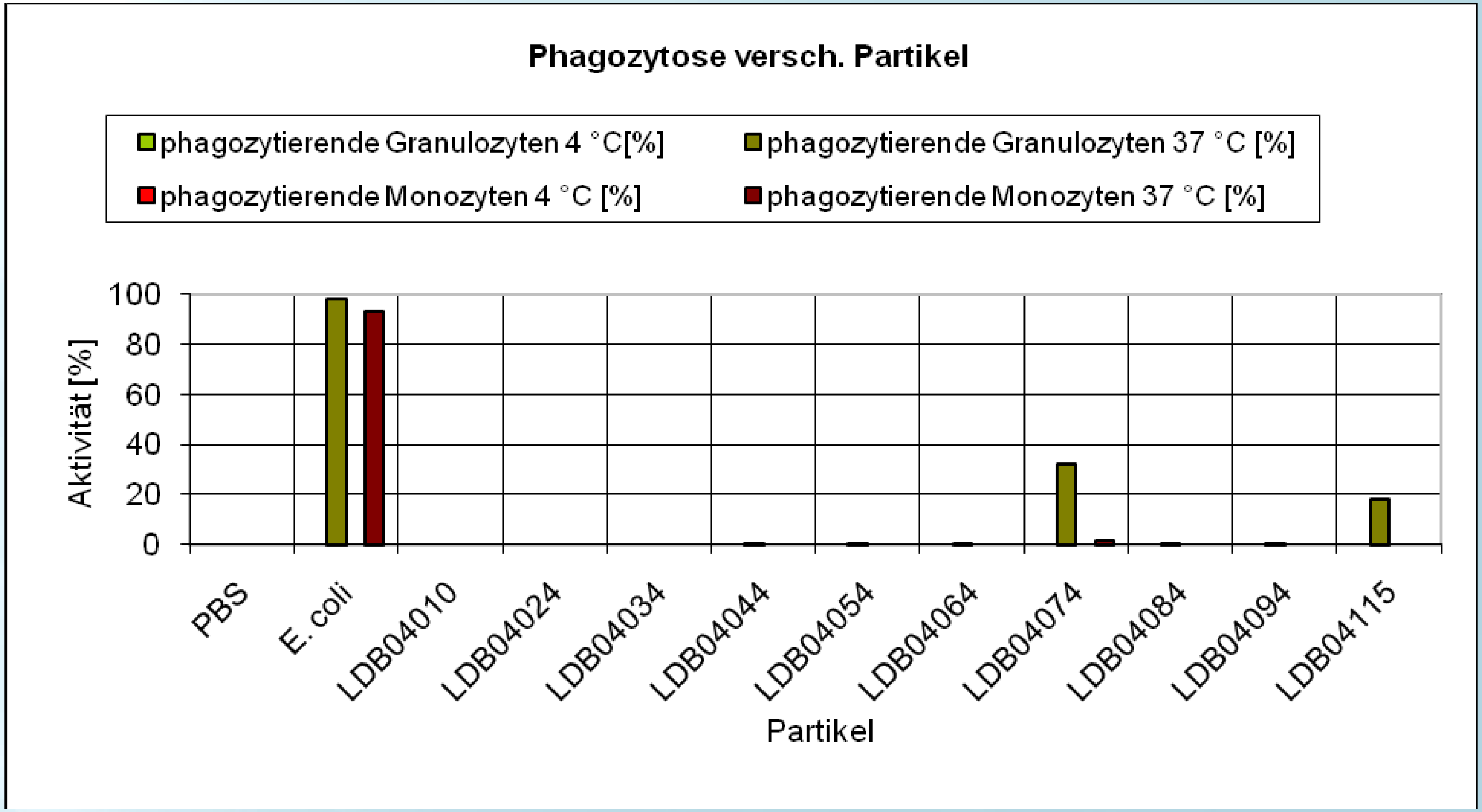
RKO Cells 24 h in Suspension 20 g/l Particles (diameter 1 µm) with slow movement (no abrasion)



Phagocyte-test



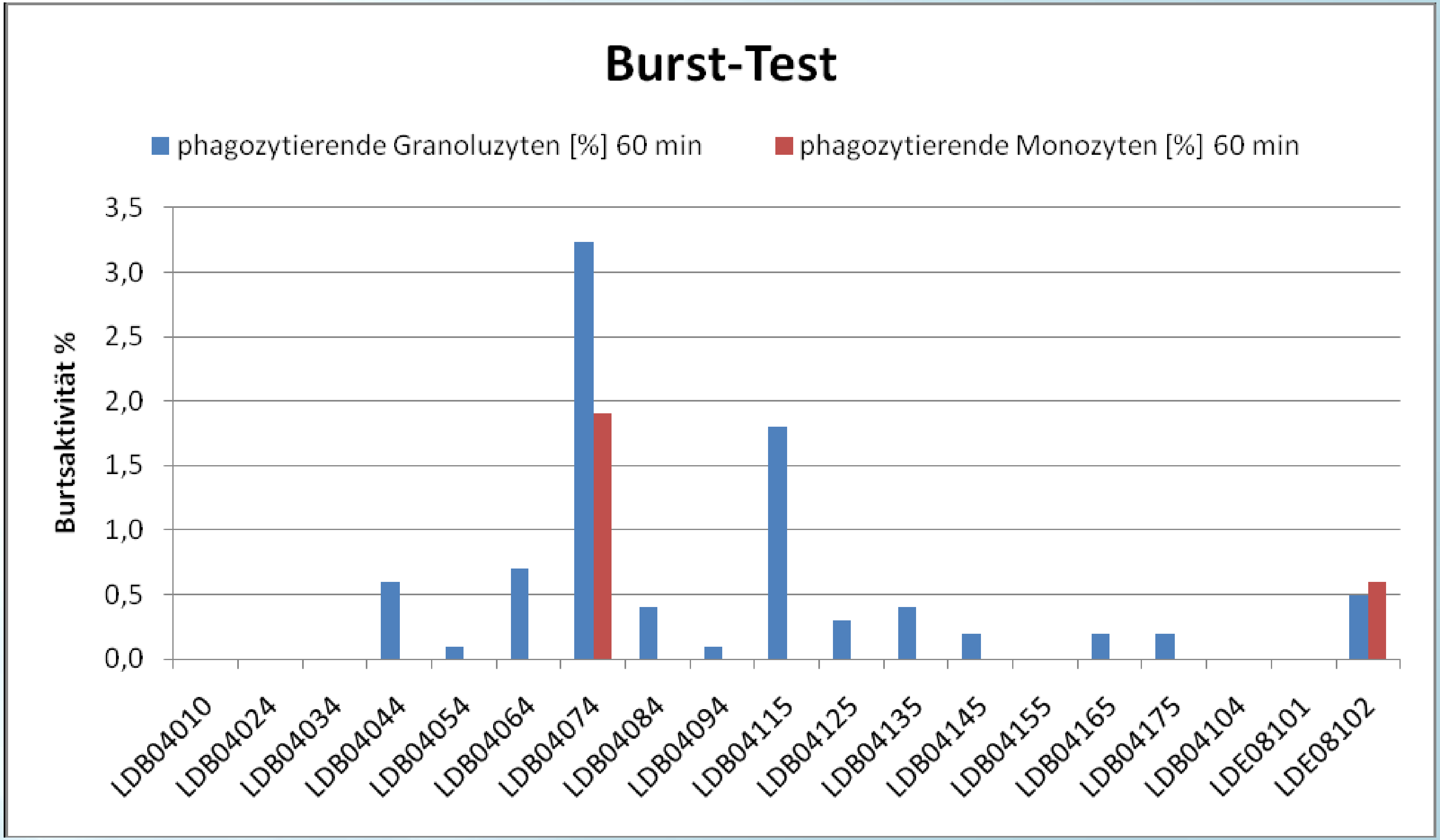
CLSM image
4.3 µm silica
coated with different
Polyelectrolytes
40 µm x 40 µm



074: PAH/PSS/PAH/Hyaluronic acid

115: PAH/PSS/PAH/PSS/PAH (cationic)

R. Georgieva, H. Bäumler, Inst. Transfusion Medicine
Charite Berlin



Again Hyaluronic acid, PAH and Aminoguanidine largest activity

R. Georgieva, H. Bäumler, Inst. Transfusion Medicine, Charite Berlin

Possible Improvements by LbL Encapsulation

Preventing radical reaction with surrounding tissue:

Phototoxicity of TiO_2 (**Anastas**, Rutil)

Radicals are captured in LbL films

Enzymatic degradation of dye pigments:

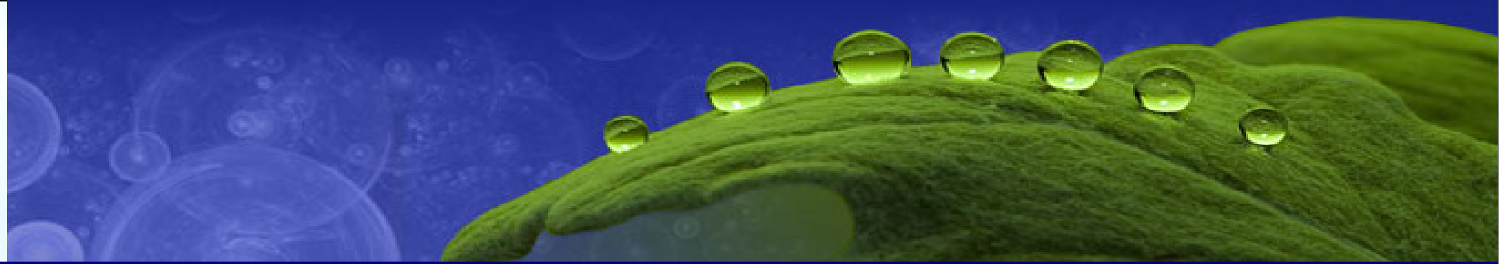
Prevention by LbL films: impermeable for enzymes

Fragmentation of pigments, release of Nanoparticles:

Nanoparticles can‘t leave the capsules; Tattoo removal might be hindered!

Bleaching:

protection by LbL hardly possible

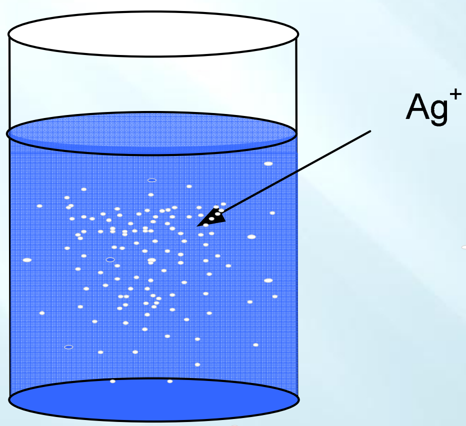


Possible Improvements by LbL Encapsulation

Prevention of poisson metal ion release?:

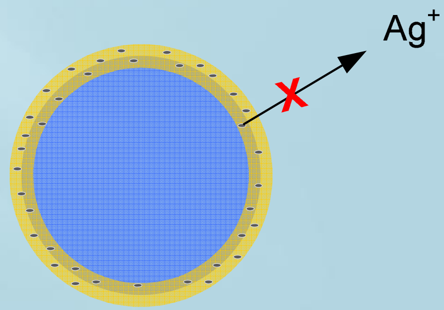
Experiment for safe immobilization of Silver nanoparticles:

- used as bacteriocides, high surface area → releasing sufficient Ag^{+} ions,
- but NP dangerous due to entering cells;
- Idea: immobilization in LbL-films?
- same concentration but no effect to bacteria



Free Ag-particles
No Cell growth

encapsulated Ag particles
Normal cell growth

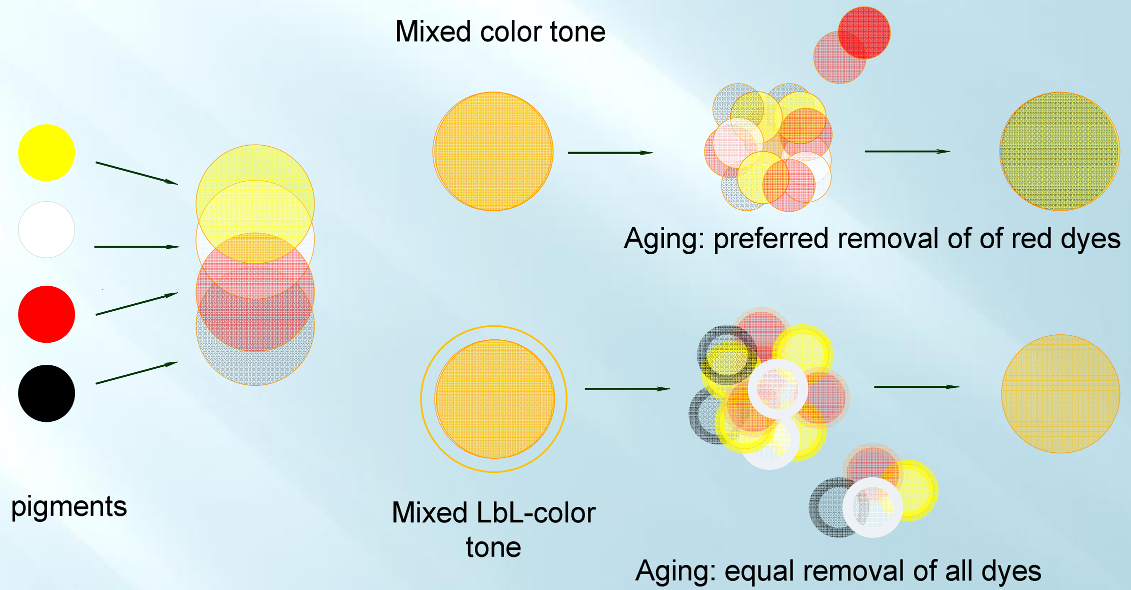


Possible mechanism
Capturing of Ag^{+} ions
Ag^{+} + PSS → Complex

Improvements by LbL Encapsulation

Recognition of immune system:

Lead to different removal from the skin (phagocytose) → change of color tones



Ongoing project with MTDerm:

uniform surface coatings, same removal rate → color tone remains

Unsolved question: Which coating gives slowest removal?

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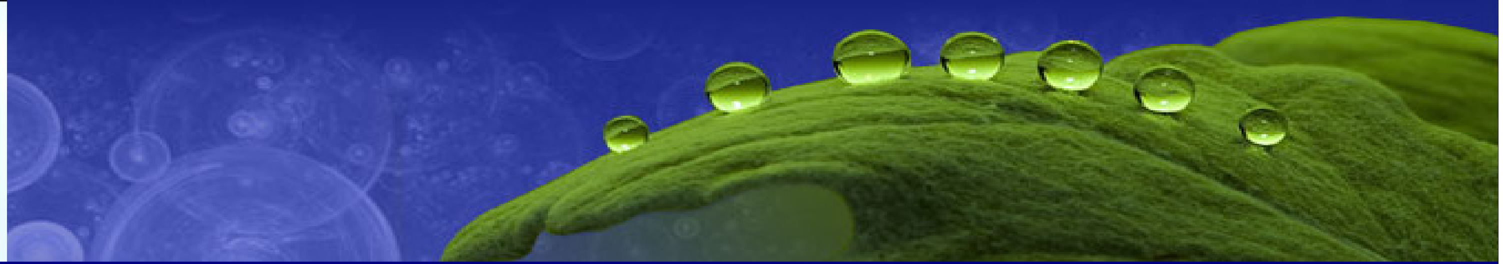
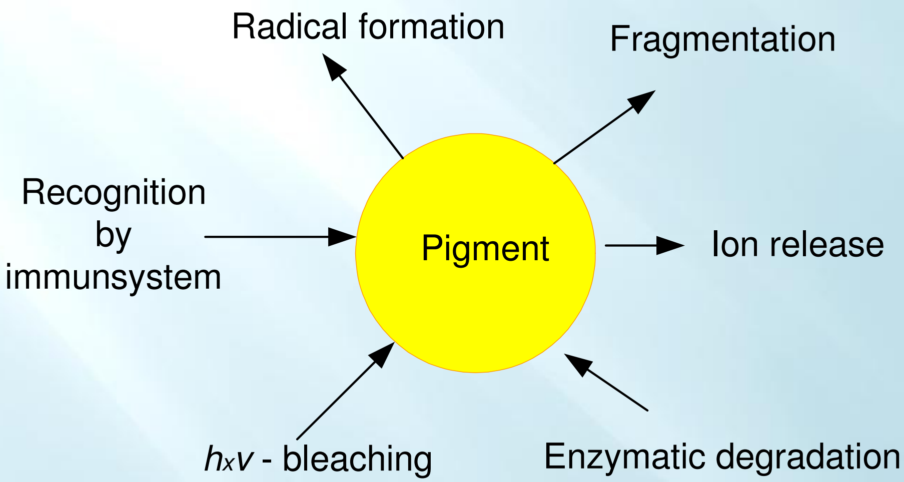


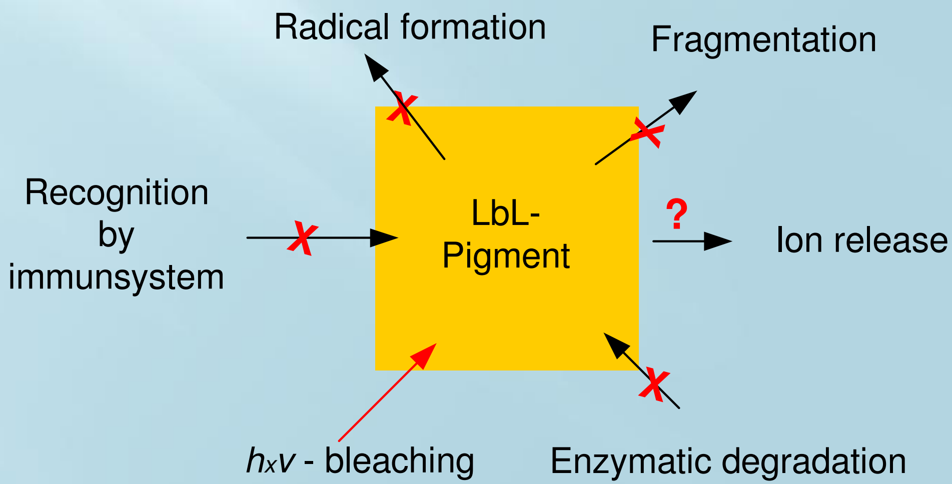
Summary

- LbL- technology can be used for pigment encapsulation;
- Several advantages for tattoo pigments:
  - prevention of allergic reactions or inflammations
  - prevention of fast removal from skin

Free pigment



Encapsulated pigment



Thanks

- Dr. Kluge, Dr. D. Lewe, MT Derm for collaboration and money
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  for Phagocytose and Burst Test

You for your attention